

Report number: 1021  
Status: waiting for approval areasupervisor  
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## 1. Specific data

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Phone Number in case of  
Emergency: 0624482558 Esengul Yildirim

Name of Experimental unit: Escherichia coli level ML1

Objective of the experimental unit: To test how Escherichia coli grows in the presence of chemicals that leak from land mines: DNT, DNB and TNT; and to be able to quantify these chemicals via inducible promoters of the bacteria

Enter the experimental approach: - DNT, DNB and TNT (called compounds from now on for simplicity) will be obtained from SIGMA Aldrich in form of a liquid solution. Acetonitrile is the solvent of the solution. These solutions come in 1-1.2 mL ampules depending on which compound. We plan to use between 3-10 ampules of 1mL of each compound (1.2mL in the case of DNT). - These compounds will be stored in their original ampule in a fire-safe cupboard before their usage. - When required to work with these compounds, Butyl-rubber gloves will be worn, with a minimum thickness of 0.3mm. Besides, the chemicals will be always handled in the fume cupboard. To avoid risks, no more than one ampule will be opened in the working space, and it will be open wearing gloves. To break the ampulle, if the Butyl-rubber gloves are not handy enough, two pairs of Nitrile-rubber gloves will be worn. The chemicals will be diluted from the ampulle (1g/L) into a range of 0-200mg/L with acetonitrile. Once the chemicals are in the desired concentration (0-200 mg/L) they will be mixed, still in the fume cupboard, with a 1X volume of bacterial solution in a 96-well plate to be analysed in the plate reader. Acetonitrile will be also used on its own as a 'blank' to correct for its effect on the growth of E.coli. This way we can compare growth with acetonitrile only against acetonitrile + DNT/DNB/TNT. The pipette tips used will be placed in an Erlenmeyer flask with water.

First Flow Chart of the Process: ()

Second Flow Chart of the Process: ()

Third Flow Chart of the Process: ()

First Plan of the Laboratory: ()

Second Plan of the Laboratory: ()

Third Plan of the Laboratory: ()

Location of the experimental unit: Biotechnology building, biocatalysis department

Area supervisor: Esengul Yildirim

Committee for Safety, Health, TNW-BT

Welfare and Environment:

Head of section/group leader: Fred Hagen







Head of department: Isabel Arends

## 2. Chemicals, gases, biologicals

### Chemicals

CAS NR: 121-14-2  
Name: 2,4-DINITROTOLUENE  
Quantity: 10 ampules of 1mL







#### Chemwatch hazard ratings

| 2,4-DINITROTOLUENE |   | ADR class: 6.1   |  |
|--------------------|---|--|--|
|                    |   |  |  |
| Flammability       | 1 |   |  |
| Toxicity           | 3 |   |  |
| Body Contact       | 3 |   |  |
| Reactivity         | 2 |   |  |
| Chronic            | 3 |   |  |

Nanomaterial: No  
CMR agent: No  
Hazards and Precautions: Classification according to Regulation (EC) No 1272/2008  
Carcinogenicity (Category 1B), H350 Germ cell mutagenicity (Category 2), H341 Reproductive toxicity (Category 2), H361f Acute toxicity (Category 3), H331 Acute toxicity (Category 3), H311 Acute toxicity (Category ), H301 Specific target organ toxicity -repeated exposure (Category 2), H373 Chronic aquatic toxicity (Category 2), H411 ----- 1000 ug/mL in acetonitrile The final maxim concentration will be 0,2mg/mL;

CAS NR: 75-05-8  
Name: ACETONITRILE  
Quantity: 3 ampules of 1mL

#### Chemwatch hazard ratings







| ACETONITRILE |   | ADR class: 3   |  |
|--------------|---|--|--|
|              |   |  |  |
| Flammability | 3 |   |  |
| Toxicity     | 2 |   |  |
| Body Contact | 2 |   |  |
| Reactivity   | 1 |   |  |
| Chronic      | 3 |   |  |

Nanomaterial: No  
CMR agent: No  
Hazards and Precautions: ADR: 34mg/m3 Classification according to Regulation (EC) No

1272/2008 Flammable liquids (Category 2), H225 Acute toxicity, Oral (Category 4), H302 Acute toxicity, Inhalation (Category 4), H332 Acute toxicity, Dermal (Category 4), H312 Eye irritation (Category 2), H319 This product will be handled with gloves. Gloves must be inspected prior to use. A proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product will be used. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it. Butyl rubber gloves will be used!

CAS NR: 99-65-0  
 Name: 1,3-Dinitrobenzene solution  
 Quantity: 3 ampules of 1mL




**Chemwatch hazard ratings**




| M-DINITROBENZENE |   | ADR class: 6.1  |  |
|------------------|---|---|--|
|                  |   |   |  |
| Flammability     | 1 |   |  |
| Toxicity         | 4 |  |  |
| Body Contact     | 4 |  |  |
| Reactivity       | 2 |  |  |
| Chronic          | 2 |  |  |

Nanomaterial: No  
 CMR agent: No  
 Hazards and Precautions: Classification according to Regulation (EC) No 1272/2008 Acute toxicity, Inhalation (Category 2), H330 Acute toxicity, Dermal (Category 1), H310 Acute toxicity, Oral (Category 2), H300 Specific target organ toxicity- repeated exposure (Category 2), H373 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410 ADR Iclass: 34mg/m3 The final maxim concentration will be 0,2mg/mL;

CAS NR: 118-96-7  
 Name: 2,4,6 - Trinitrotoluene - 15N3  
 Quantity: 3 amuples of 1mL

**Chemwatch hazard ratings**

| TRINITROTOLUENE (TNT) |   | ADR class: 1.1D  |  |
|-----------------------|---|--|--|
|                       |   |  |  |
| Flammability          | 1 |   |  |
| Toxicity              | 3 |   |  |

|              |   |   |
|--------------|---|---|
| Body Contact | 3 |    |
| Reactivity   | 3 |   |
| Chronic      | 2 |  |

|                          |  |
|--------------------------|--|
| Nanomaterial:            | No   |
| CMR agent:               | No   |
| Hazards and Precautions: | Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP] Acute toxicity, Oral (Category 4) Acute toxicity, Inhalation (Category 2) Acute toxicity, Dermal (Category 3) Specific target organ toxicity -repeated exposure (Category 2) Chronic aquatic toxicity (Category 2) ADR class 34mg/m3 ----- The final maxim concentration will be 0,2mg/mL; ----- *General comment: in the iGEM office we have a printed version of all the safety sheets of the chemicals we are going to use in the lab, namely ( 2,4,6 - Trinitrotoluene - 15N3 , 1,3-Dinitrobenzene solution , acetonitrile and 2,4-Dinitrotoluene solution). Before entering the lab, each student of the team have to read first these documents and to be aware about all the safety measures required and potential risks |

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## Biologicals

|                |                  |
|----------------|------------------|
| Name:          | Escherichia coli |
| Quantity:      |                  |
| Type/category: | Micro organism   |

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## Miscellaneous

### 3 Equipment

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#### Equipment

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Number:

Name: Small cultivation Erlenmeyer flask

Description: Small cultivation Erlenmeyer flask

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#### Health risks & safety measure(s)

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Number:

Name: 96-well plate

Description: 96-well plate

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#### Health risks & safety measure(s)

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Number:

Name: Fume Cupboard

Description: Portable shaker water incubator will be placed in the fume cupboard. A fume cupboard is a piece of laboratory equipment designed to limit exposure to dangerous fumes. The air inside the fume hood is either vented to the outside or else filtered and recirculated.

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#### Health risks & safety measure(s)

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Number:

Name: Plate reader

Description: Device to measure the OD and fluorescence emission of the cells in time

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**Health risks & safety measure(s)**

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Number:

Name:

Description:

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**Health risks & safety measure(s)**

**Additional information**

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**Miscellaneous:**

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## 4 Failures & exceptional conditions

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### Failure/condition

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|                    |   |
|--------------------|---|
| Failure/condition: | Shut down procedure   |
| Safety measure(s): | <p>- If some chemical is spilled on the gloves, they will be removed as soon as possible without touching the skin with the gloves. -In case of emergency (eg. spills) the affected area must be covered with tissues. The room needs to be evacuated for half an hour. When leaving the room make sure you take off and dispose properly the lab coat. When returning in the room, plastic bags for biological waste need to be taken. The waste should be disposed in a chemical waste bin. The Biosafety officer needs to be contacted for reporting the situation</p> |

### Miscellaneous:

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## 5 Emergency Card

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### Description of emergency shutdown

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In case of emergency (eg. spills) the affected area must be covered with tissues. The room needs to be evacuated for half an hour. When leaving the room make sure you take off and dispose properly the lab coat. When returning in the room, plastic bags for biological waste need to be taken. The waste should be disposed in a chemical waste bin. The Biosafety officer needs to be contacted for reporting the situation.

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## 6 Ergonomics

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**Ergonomic risks:**

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**Miscellaneous:**

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## 7 Disposal and Waste

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### Used/produced chemicals

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#### 2,4-DINITROTOLUENE

|                  |  |
|------------------|--|
| Quantity:        | 10 ampules of 1mL  |
| Way of disposal: | - The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed |
| Estimated costs: |  |

#### ACETONITRILE

|                  |  |
|------------------|--|
| Quantity:        | 3 ampules of 1mL   |
| Way of disposal: | - The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed |
| Estimated costs: |  |

#### 1,3-Dinitrobenzene solution

|                  |  |
|------------------|--|
| Quantity:        | 3 ampules of 1mL   |
| Way of disposal: | - The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed |
| Estimated costs: |  |

#### 2,4,6 - Trinitrotoluene - 15N3

|                  |  |
|------------------|--|
| Quantity:        | 3 amuples of 1mL   |
| Way of disposal: | - The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed |
| Estimated costs: |  |

## Used/produced biologicals

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### Escherichia coli

Quantity:

Way of disposal:

- The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed

Estimated costs:

## Used equipment

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### Small cultivation Erlenmeyer flask

Way of disposal:

- The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed

Estimated costs:

### 96-well plate

Way of disposal:

- The LB with E.coli and each independent compound (DNT, DNB or TNT) will be collected in an independent bottle (depending on with compound is used); and labelled accordingly. The bacteria will be killed by adding an antibacterial agent for the required time. Afterwards, the bottles will be brought to the Chemical Central Waste to be disposed

Estimated costs:

### Fume Cupboard

Way of disposal:

Estimated costs:

### Plate reader

Way of disposal:

Estimated costs:

Way of disposal:

Estimated costs: